

FIG. 1

	10	20	30	40	50
Bet v 1 sense	5'-	A A T T A T G A G A C T G A G A C C A C C T C T G T T A T C C C A G C A G C T C G	-3'		
Bet v 1 non-sense	3'-	T T A A T A C T C T G A C T C T G G I G G A G A C A A T A G G G T C G T C G A G C	-5'		
sense primer	5'-	T G A G A C C C C C C T C T G T T A T C C C A G			
non-sense primer	3'-	A T A C T C T G A C T C T G G G G G A G A C A			

FIG. 2

Oligonucleotide primers for site directed mutagenesis of Bet v 1 (No. 2801).

all sense	1: 183Bv,	15-mer 5'-	G T T G C C A A C G A T C A G
1 sense	2: 184Bv,	23-mer 5'-	T G A G A C C C C T C T G T T A T C C C A G
1 non-sense	3: 185Bv,	23-mer 5'-	A C A G A G G G G T C T C A G T C T C A T A
2 sense	4: 186Bv,	31-mer 5'-	G A T A C C C T C T T T C C A C A G G T T G C A C C C C A A G
2 non-sense	5: 187Bv,	31-mer 5'-	A C C T G T G G A A A G A G G G T A T C G C C A T C A A G G A
3 sense	6: 188Bv,	23-mer 5'-	A A C A T T T C A G G A A A T G G A G G C C
3 non-sense	7: 189Bv,	23-mer 5'-	T T C C T G A A A T T T T C A A C A C T
4 sense	8: 190Bv,	23-mer 5'-	T T A A G A A C A T C A G C T T T C C C G A A
4 non-sense	9: 191Bv,	23-mer 5'-	A G C T G A T G T T C T T A A T G G T T C C A
5 sense	10: 192Bv,	23-mer 5'-	G G A C C A T G C A A A C T T C A A A T A C A
5 non-sense	11: 193Bv,	23-mer 5'-	A G T T T G C A T G G T C C A C C T C A T C A
6 sense	12: 194Bv,	23-mer 5'-	T T T C C C T C A G G C C T C C C T T C A A
6 non-sense	13: 195Bv,	23-mer 5'-	A G G C C T G A G G G A A A G C T G A T C T T
7 sense	14: 196Bv,	24-mer 5'-	T G A A G G A T C T G G A G G G C C T G G A A C
7 non-sense	15: 197Bv,	24-mer 5'-	C C C T C C A G A T C C T T C A A T G T T T C
8 sense	16: 198Bv,	24-mer 5'-	G G C A A C T G G T G A T G G A G G A T C C A T
8 non-sense	17: 199Bv,	24-mer 5'-	C C A T C A C C A G T T G C C A C T A T C T T
all non-sense	18: 200Bv,	15-mer 5'-	C A T G C C A T C C G T A A G

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FIG. 3

1 (A-C)

GGTGTGTTTAATTATGAGACTGAGACCACCTCTGTTATCCCAGCAGCTCGACTGTTCAAG 60

G V F N Y E T E T T F S V I P A A R L F K 20

9 (A-G) 2 (A-C) 2 (A-C)

GCCTTTATCCTTGATGGCGATAACCTCTTTCCAAAGGTTGCACCCCAAGCCATTAGCAGT 120

A F I L D-G G D N-T L F P K-Q V A P Q A I S S 40

3 (GA-TC) 7 (AA-TC) 4 (G-C) 6 (GA-TC)

GTTGAAAACATTGAAGGAAATGGAGGGCCTGGAACCATTAAGAAGATCAGCTTTCCCGAA 180

V E N I E-S G N-S G G P G T I K K-N I S F P E-S 60

5 (CA-TG)

GGCCTCCCTTTCAAGTACGTGAAGGACAGAGTTGATGAGGTGGACCACACAAACTTCAAA 240

G L P F K Y V K D R V D E V D H T-A N F K 80

TACAATTACAGCGTGATCGAGGGCGGTCCCATAGGCGACACATTGGAGAAGATCTCCAAC 300

Y N Y S V I E G C P I G D T L E K I S N 100

10 (GAG-CAC) 8 (CCC-TGG)

GAGATAAAGATAGTGGCAACCCCTGATGGAGGATCCATCTTGAAGATCAGCAACAAGTAC 360

E I K I V A T P-G D G G S I L K I S N K Y 120

CACACCAAAGGTGACCATGAGGTGAAGGCAGAGCAGGTTAAGGCAAGTAAAGAAATGGGC 420

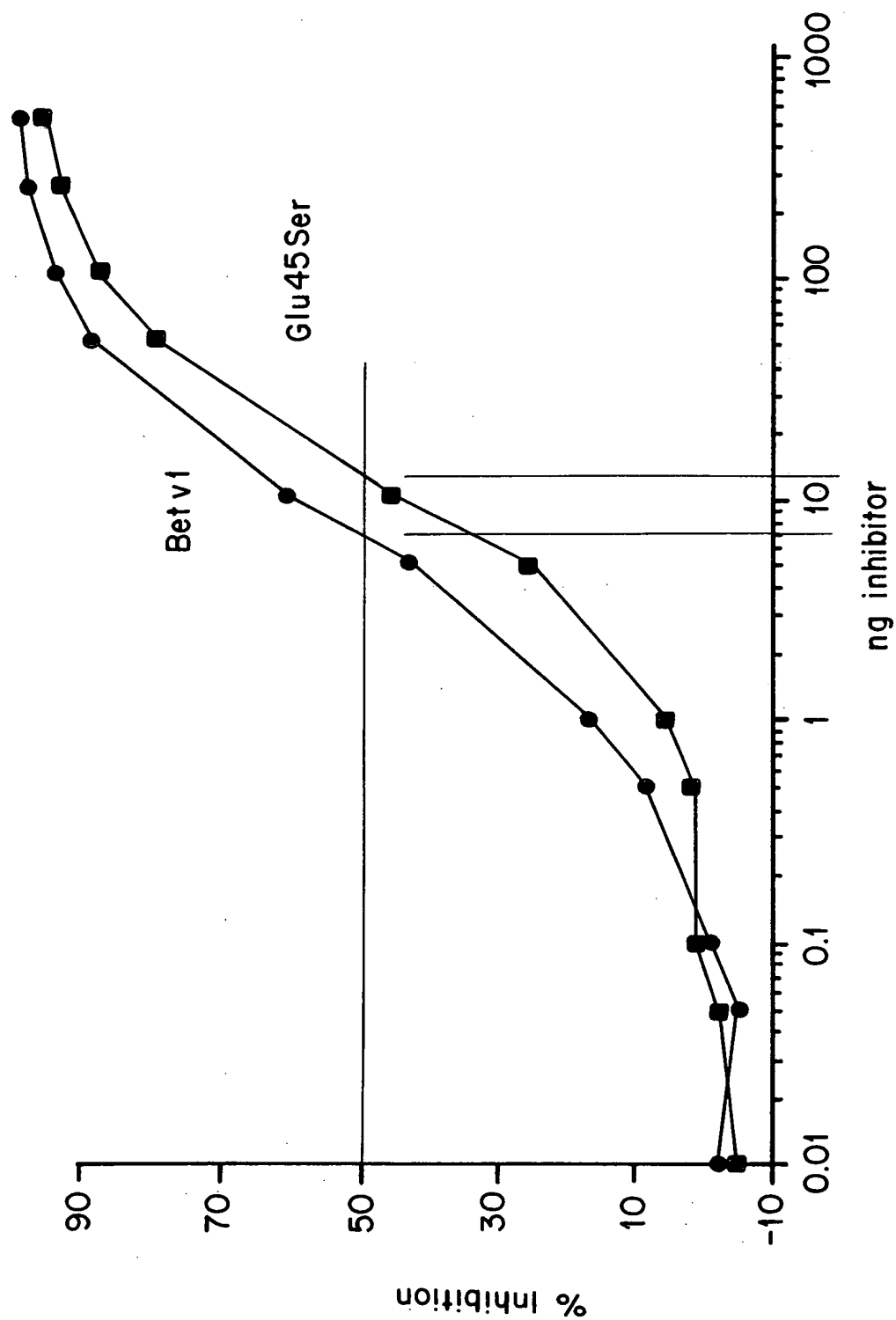
H T K G D H E V K A E Q V K A S K E M G 140

GAGACACTTTTGAGGGCCGTTGAGAGCTACCTCTTGGCACACTCCGATGCCTACAATAA 480

E T L L R A V E S Y L L A H S D A Y N stop 159

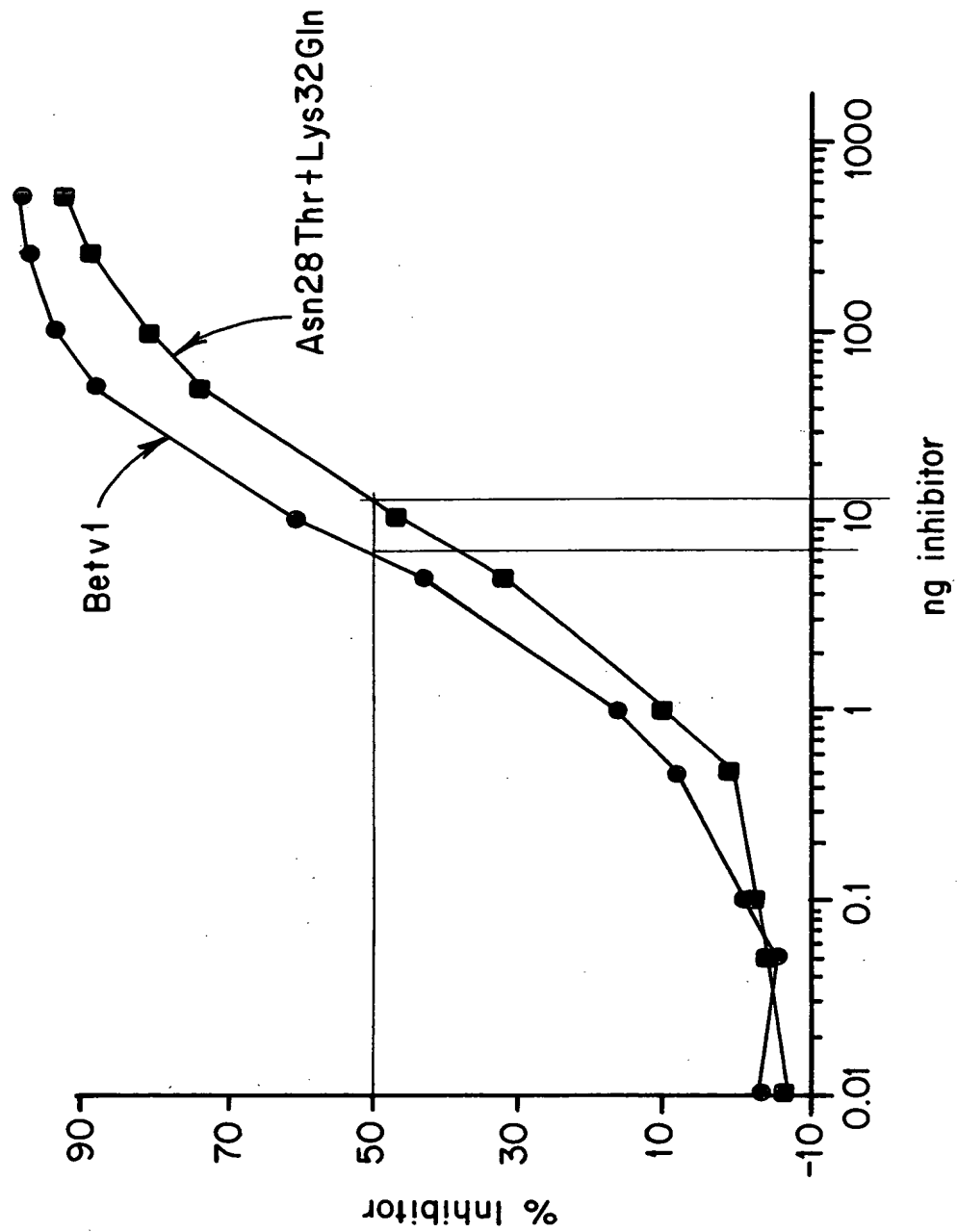
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FIG. 4



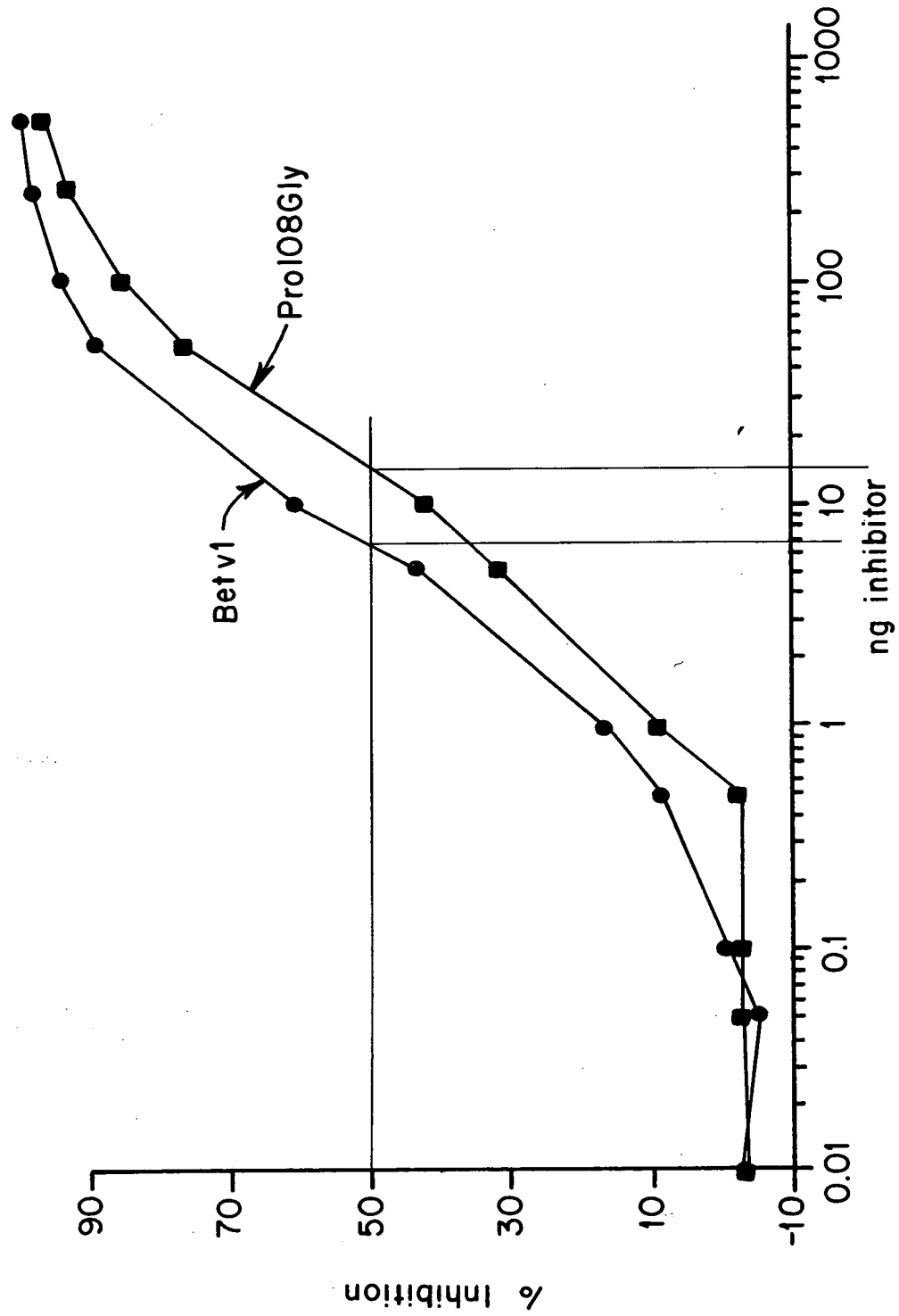
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FIG. 5



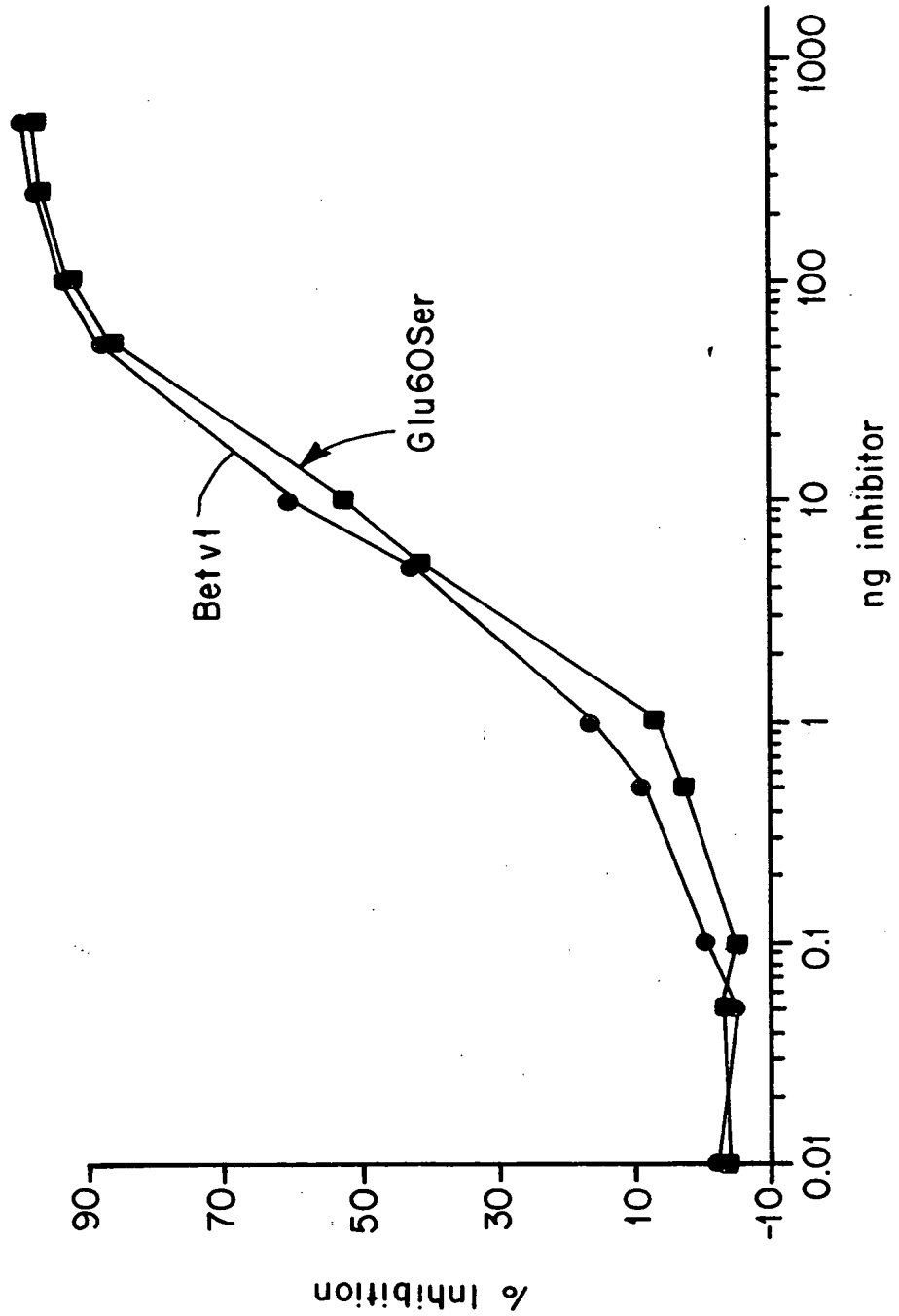
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FIG. 6



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FIG. 7



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FIG. 8A

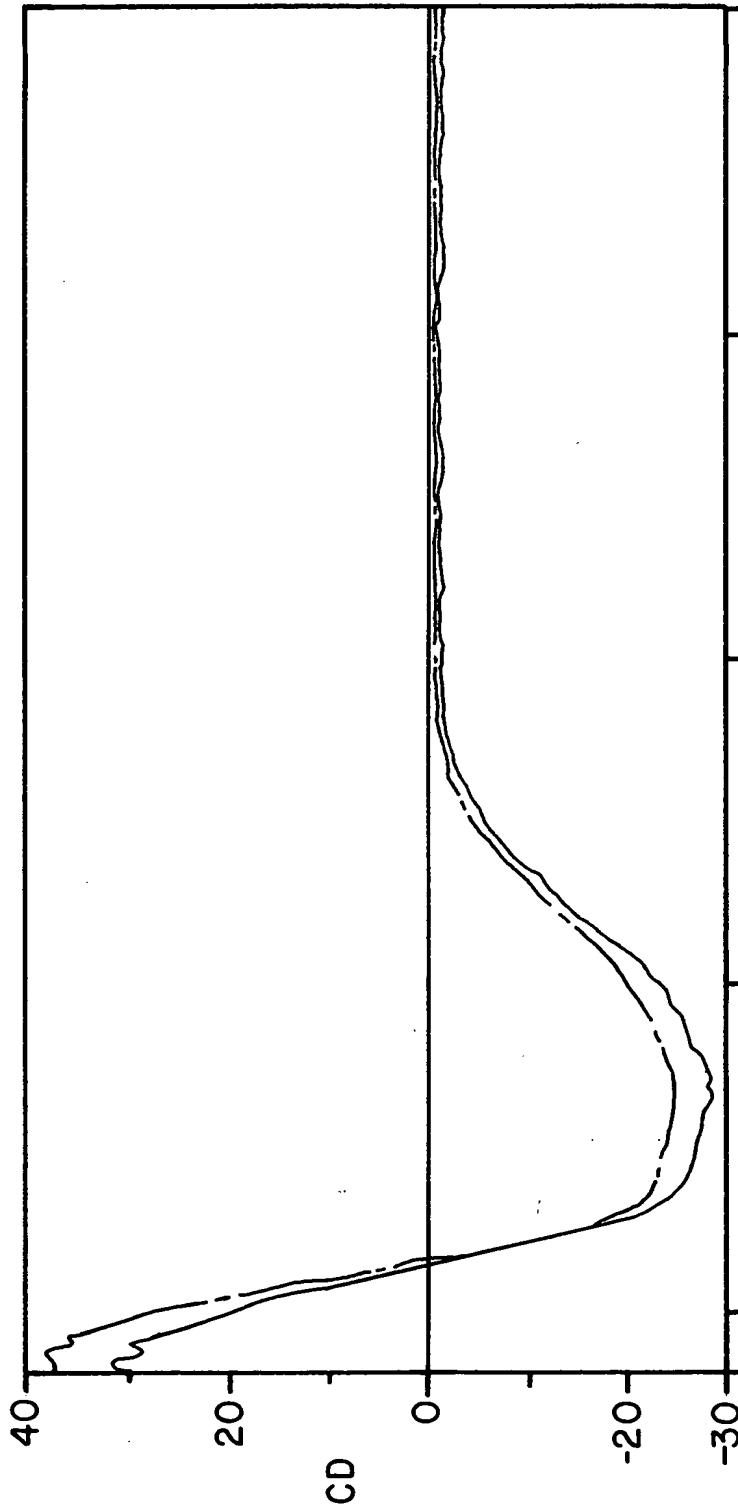
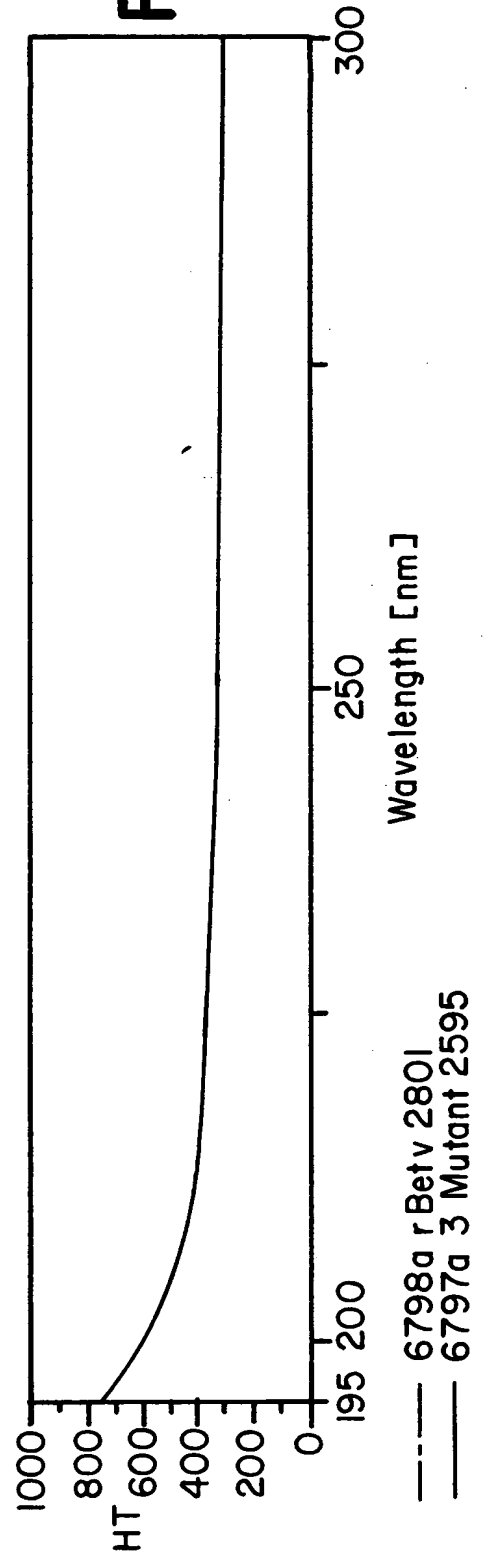
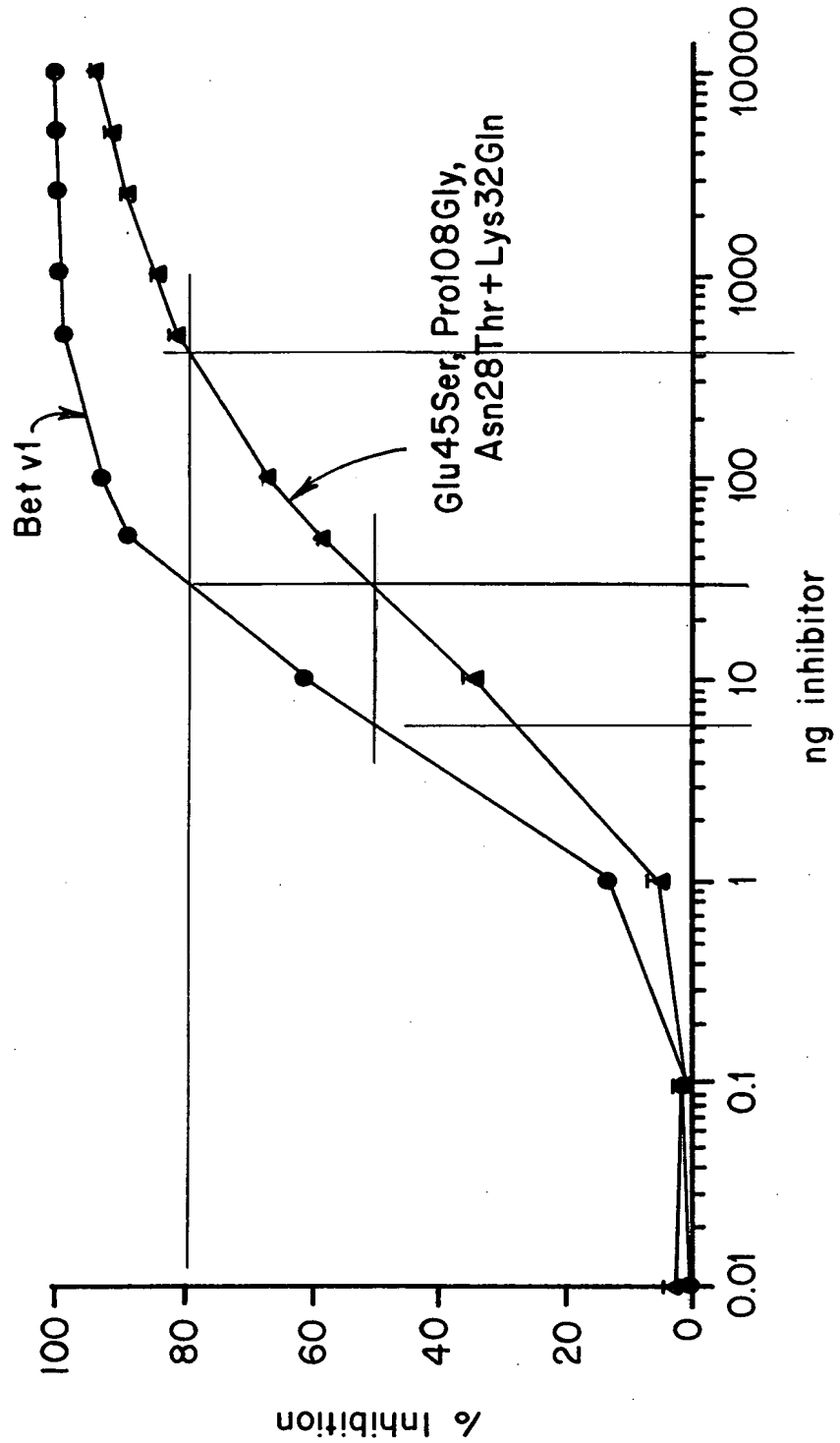


FIG. 8B



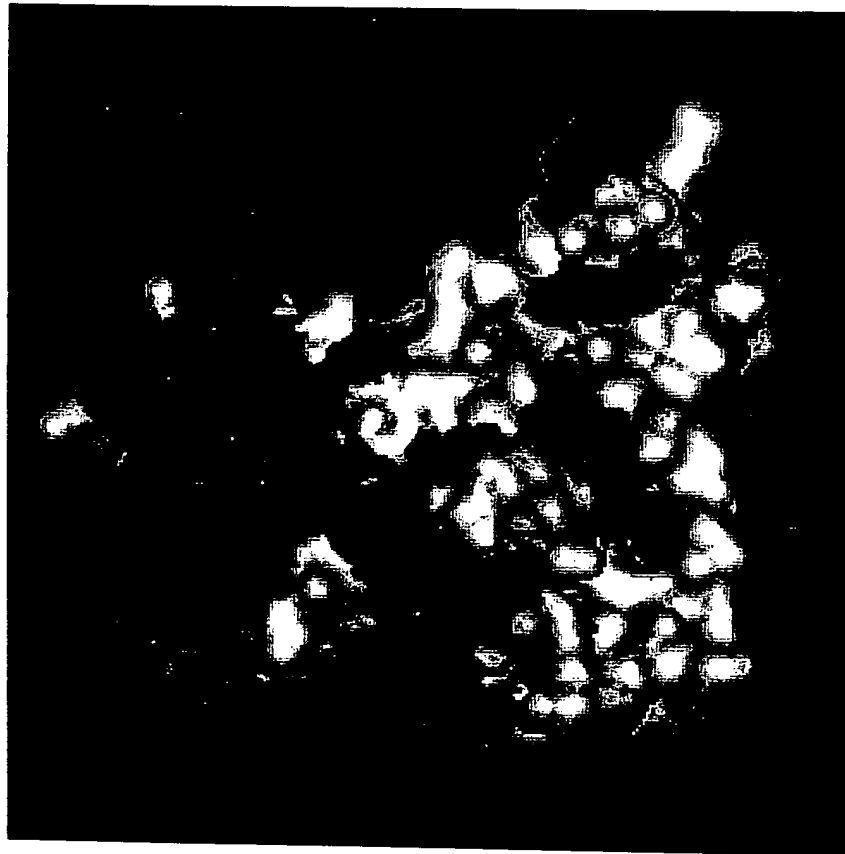
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FIG. 9



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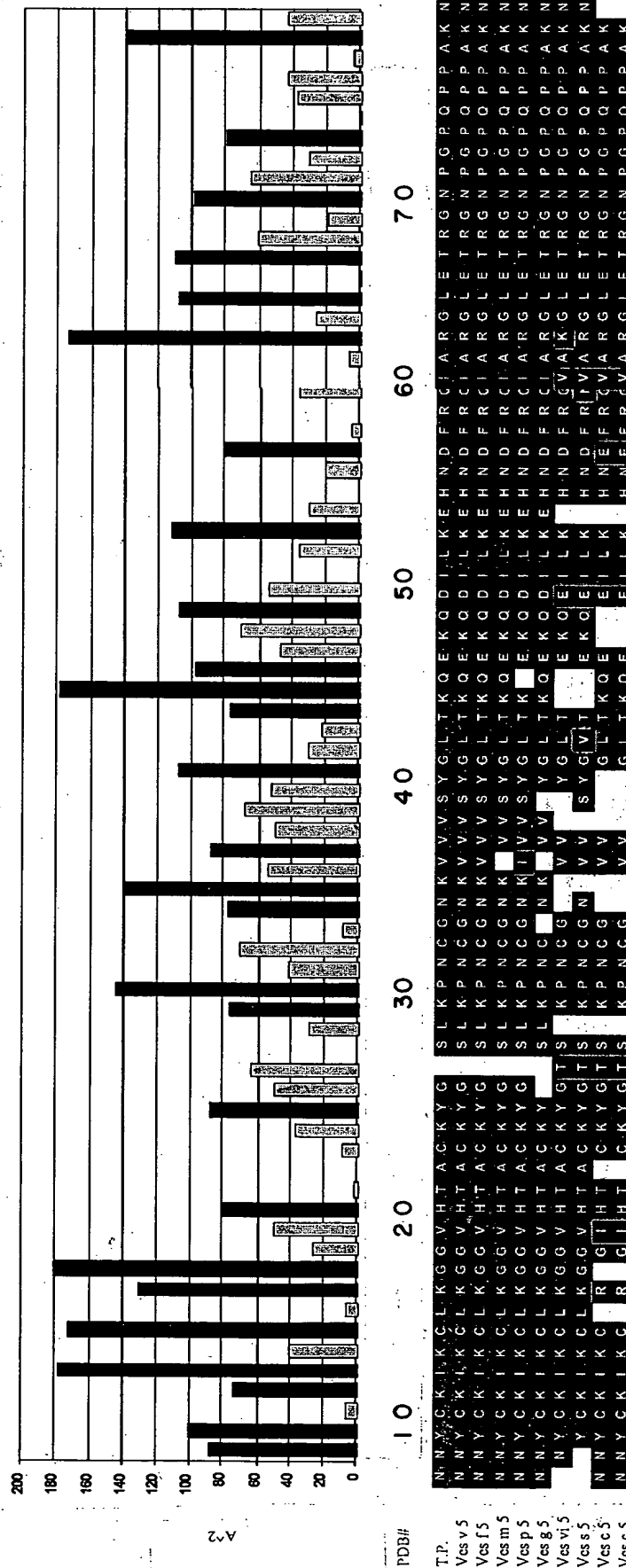
FIG. 10A



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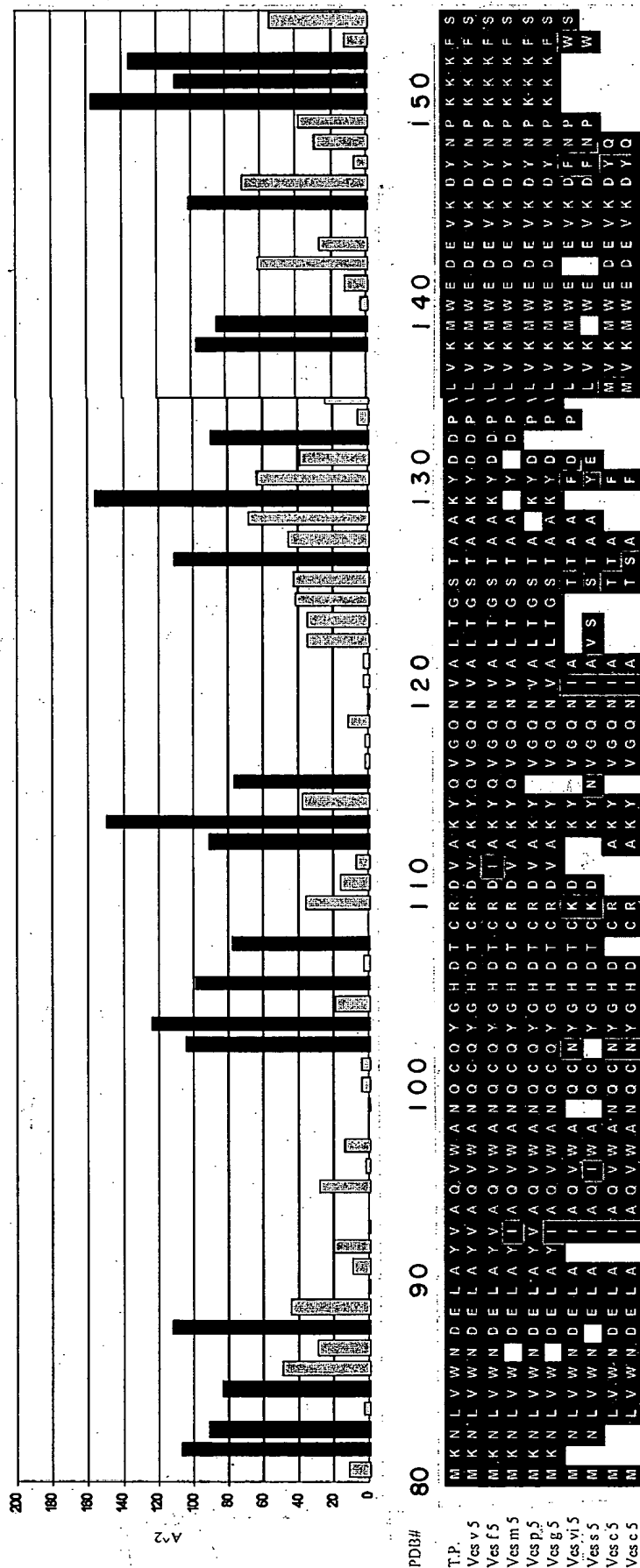
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FIG. 10B



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FIG. 10C



[illegible]

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FIG. 11

Mutant-specific oligonucleotide primers used for Ves v 5 mutants.
Mutated nucleotides underlined.

Ves v 5 mutant 1 (K72A)

Ves v 5 sense	5'-	<u>A</u> CCACAGCCCTCCAGCGAAGAAATATGAAATAATTTGGTATGGA	3'
Ves v 5 non-sense	3'-	TGGTGTCGGAGGTCGCTTCTTATACCTTTTAAACCATACCT	5'
sense primer	5'-	CCAGCGGCTAATATGAAATA	3'
non-sense primer	3'-	GTCGGAGGTCGCCGATTATAC	5'

Ves v 5 mutant 2 (Y96A)

Ves v 5 sense	5'-	GGCTAATCAATGTCAAATATGGTCAACGATACTTGCAAGGATG	3'
Ves v 5 non-sense	3'-	CCGATTAGTTACAGTTATACCAAGTGCTATGACGTCCTAC	5'
sense primer	5'-	TGTCAAAGCTGTCACGATACT	3'
non-sense primer	3'-	TTAGTTACAGTTCGACCATG	5'

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FIG. 12

Oligonucleotide primers for the site directed mutagenesis of Ves v 5:

all sense	1 : XhoI start,	38-mer:
	EcoRI	
		5'-C C G C T C G A G A A A G A A C A A T T A T T G T A A A A T G
	L E K	N Y C K I K
	KaX2 cleavage site	amino terminus of Ves v 5
1 sense	1 : K72As	5'-C C A G C G G C T A A T A T G A A A A T
1 non-sense	2 : K72Aa	5'-C A T A T T A G C C G C T G G A G G C T G
2 sense	3 : Y96As	5'-T G T C A A G C T G G T C A C G A T A C T
2 non-sense	4 : Y96Aa	5'-G T G A C C A G C T T G A C A T T G A T T
all non-sense	7 : CT-pPICZaA, 21-mer	5'-A T T C A T C A G C T G C G A G A T A G G

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FIG. 13

1	AACAATTATTGTAAAATAAAATGTTTGAAAGGAGGTGTCCATACTGCCTGCAAATATGGA	60
1	N N Y C K I K C L K G G V H T A C K Y G	20
61	AGTCTTAAACCGAATTGCGGTAATAAGGTAGTGGTATCCTATGGTCTAACGAAACAAGAG	120
21	S L K P N C G N K V V V S Y G L T K Q E	40
121	AAACAAGACATCTTAAAGGAGCACAATGACTTTAGACAAAAAATTGCACGAGGATTGGAG	180
41	K Q D I L K E H N D F R Q K I A R G L E	60
1 (K 72A) (AAG-GCT)		
181	ACTAGAGGTAATCCTGGACCACAGCCTCCAGCGAAGAATATGAAAAATTTGGTATGGAAC	240
61	T R G N P G P Q P P A K N M K N L V W N	80
2 (Y9 6A) (TA-GC)		
241	GACGAGTTAGCTTATGTCGCCCAAGTGTGGGCTAATCAATGTCAATATGGTCACGATACT	300
81	D E L A Y V A Q V W A N Q C Q Y G H D T	100
301	TGCAGGGATGTAGCAAAATATCAGGTTGGACAAAACGTAGCCTTAACAGGTAGCACGGCT	360
101	C R D V A K Y Q V G Q N V A L T G S T A	120
361	GCTAAATACGATGATCCAGTTAAACTAGTTAAATGTGGGAAGATGAAGTGAAAGATTAT	420
121	A K Y D D P V K L V K M W E D E V K D Y	140
421	AATCCTAAGAAAAAGTTTTTCGGGAAACGACTTTCTGAAAACCGGCCATTACACTCAAATG	480
141	N P K K K F S G N D F L K T G H Y T Q M	160
481	GTTTGGGCTAACACCAAGGAAGTTGGTTGTGGAAGTATAAAATACATTCAAGAGAAATGG	540
161	V W A N T K E V G C G S I K Y I Q E K W	180
541	CACAAACATTACCTTGTATGTAATTATGGACCCAGCGGAAACTTTAAGAATGAGGAACTT	600
181	H K H Y L V C N Y G P S G N F K N E E L	200
601	TATCAAACAAAGTAA	612
201	Y Q T K stop	204

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FIG. 14

